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Susceptibility, Preference, and Suitability of *Carpinus* and *Ostrya* Taxa for Gypsy Moth Larvae (Lepidoptera: Lymantriidae)

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Abstract

Twenty *Carpinus* taxa and three *Ostrya* taxa, growing in the tree breeding production area at The Morton Arboretum, Lisle, IL, were evaluated in laboratory bioassays for feeding susceptibility, preference, and suitability for gypsy moth (*Lymantria dispar* (L.)) (Lepidoptera: Lymantriidae) larvae. No-choice and multiple-choice laboratory feeding studies revealed that *C. coreana*, *C. fargesii*, *C. laxiflora*, and the hybrid *C. caroliniana* × *C. orientalis* were the least suitable for larval development and pupation, and were less preferred by gypsy moth larvae. Suitability rankings for gypsy moth larval development time were highly correlated with larval longevity, but the proportion of larvae pupating was not correlated with either larval longevity or with larval development time. Pupal fresh weights were not correlated with either larval longevity or with larval development time. However, larval dry frass weights were correlated with the proportion of larvae pupating, but not correlated with pupal fresh weights. Leaf toughness and leaf thickness do not appear to be factors in gypsy moth larval suitability and preference.

Keywords: Susceptibility, preference suitability, gypsy moth, *Lymantria dispar*, *Carpinus, Ostrya*

Since its introduction into the United States in the mid-19th century, the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) is still considered one of the most destructive and persistent pest of nursery crops, landscape plants, and rural and urban forest trees. Gypsy moth larvae feed on over 500 species of woody plant (Forbush and Fernald 1896, Liebhold et al. 1995) preferring oaks (*Quercus* spp.) (Barbosa and Krischik 1987), and other hardwood species (Elkinton and Liebhold 1990, Shields et al. 2003). Chronic defoliation can lead to trees that may be predisposed to abiotic factors such as drought, and biotic factors including lethal secondary wood-boring insects and pathogens, for example, the two-lined chestnut borer (*Agrilus bilineatus*) (Weber) and pathogens such as Armillaria root rot (*Armillaria mellea*). Common to most woody landscape and forest trees, host plant resistance for *L. dispar* has historically been compiled from anecdotal field studies and observations (Forbush and Fernald 1896, Mosher 1915) and while helpful, these studies require some level of interpretation as for host susceptibility, preference, and suitability. More recently, Liebhold et al. (1995) ranked over 600 North American angiosperm and gymnosperm tree species as to their susceptibility to the gypsy moth based on previous field and laboratory tests.

These susceptibility and suitability rankings were based on gypsy moth defoliation, larval abundance, growth and survival, larval foliage preference, pupal weights, and ratio of pupal weight on host to the pupal weight on white oak (*Q. alba*) (standard host) (Liebhold et al. 1995). Concurrently, extensive studies have focused on the relative susceptibility and suitability of black oak (*Q. velutina*), bur oak (*Q. macrocarpa*), cherrybark oak (*Q. pagoda*), northern red oak (*Q. rubra*), pin oak (*Q. palutris*), swamp white oak (*Q. bicolor*), white oak (*Q. alba*), willow oak, (*Q. phellos*), northern pin oak (*Q. ellipsoidalis*), southern red oak (*Q. falcata*), water oak (*Q. nigra*), chestnut oak (*Q. prinus*), post oak (*Q. stellata*), sessile oak (*Q. petraea*), Turkey oak (*Q. cerris*), Hungarian oak (*Q. frainetto*), and Garry oak (*Q. garryana*) because of the importance of the genus in the ecology and sustainability of eastern and mid-western North American and European forests (Barbosa and Capinera 1977; Barbosa and Greenblatt 1979; Barbosa et al. 1983; Lechowicz and Jobin 1983; Mauffette et al. 1983; Lechowicz and Mauffette 1986; Miller and Hanson 1989a,b; Miller et al. 1991; Montgomery 1991; Davidson et al. 1999; Foss and Rieske 2003; Rieske et al. 2003; Shields et al. 2003; Miller 2008; Milanovic et al. 2014). However, of the over 600 tree species listed in Liebhold et al. (1995), only

Carpinus caroliniana, and *Ostrya virginiana* are listed with suitability rankings of 2 and 1, respectively. Additionally, Mauffette et al. (1983) found *O. virginiana* to be strongly preferred in Quebec compared to New England where it is considered an intermediate host.

With the recent accessibility of China to foreign plant exploration, a wide variety of Asiatic woody plant material, including *Carpinus* and *Ostrya* taxa, has been discovered and developed for possible use in tree breeding programs and for increasing plant diversity in urban forests and landscapes (Ware 1992, 1995). With the recent loss of millions of North American ash (*Fraxinus* spp.) trees to the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire), and maples (*Acer* spp.) and other hardwoods to the Asian long-horned beetle (ALB) (*Anoplophora glabripennis* (Motschulsky)), there is an important and critical need for the development, and availability of new urban tree species for replanting in EAB and/or ALB affected areas, and to minimize the economic impact on communities and municipalities for the need for chemical protection from gypsy moth defoliation (Poland and McCullough 2006, Raupp et al. 2006, Sydnor et al. 2007, Kovacs et al. 2010, Sadof et al. 2011, Vannatta et al. 2012, Hauer and Peterson 2017, Herms et al. 2019). With the exception of the aforementioned North American species of *C. caroliniana* and *O. virginiana* (Liebhold et al. 1995), to the best of our knowledge, no studies have been conducted on the relative susceptibility, preference, and suitability of recently acquired and developed *Carpinus* and *Ostrya* taxa of North American, European, and Asian parentage for the gypsy moth. Here, we report the results of a study to determine the relative susceptibility, feeding preference, and suitability of *Carpinus* and *Osytra* taxa in no-choice and multiple-choice laboratory feeding bioassays for gypsy moth larvae. Results from this study will contribute to the use of additional taxa in landscape and urban forest plantings, which will minimize the need for application of chemical insecticides, and contribute to more diverse landscapes and urban forest settings.

Materials and Methods

No-choice (NC) laboratory larval feeding trials. No-choice larval feeding trials were conducted using newly hatched first instar gypsy moth larvae. Twenty *Carpinus* taxa and three *Ostrya* taxa were evaluated for relative susceptibility, larval feeding preference, and suitability for larval development (refer to Table 1 for a listing of *Carpinus* and *Osytra* taxa tested). Candidate *Carpinus* and *Ostrya* taxa were growing in The Morton Arboretum (TMA) tree breeding nursery production area at Lisle, IL, and ranged in height from 2 to 4 m and 8 to 10 cm dbh.

For the laboratory no-choice (NC) and multiple choice (MC) bioassay feeding tests, fully developed *Carpinus* leaves were randomly selected from the terminal 15 cm of each of four mid-canopy branches per tree from all four cardinal directions. The leaves were brought back to the entomology lab and held in cold storage in plastic bags at 5 °C for a maximum of 2 d. Leaves from each single tree replicate were combined for the laboratory bioassays. Depending on tree availability, one to three individual trees (replicates) each of either *Carpinus* or *Ostrya* taxon were evaluated. Bur oak (*Q. macrocarpa*) a highly preferred host of the gypsy moth, served as the standard.

First instar gypsy moth larvae, used in the NC and MC laboratory feeding studies, were reared from overwintering egg masses collected in late spring from infested trees on the grounds of TMA. Prior to use, the egg masses were held in brown Kraft paper bags in a refrigerator at approximately 4 °C. The egg masses were then placed in an incubator under a photoperiod of 16:8 (L:D) at approximately 25 °C. Within 24 hours of hatching, a cohort of 10 larvae was randomly selected and placed in a one liter (one quart) plastic container, with a screened lid to allow for ventilation, with foliage from the test *Carpinus* or *Ostrya* taxon. The containers were placed in plastic trays and were held in an incubator under a photoperiod of 16:8 (L:D) at approximately 25 °C. The containers were examined daily for larval mortality, evidence of feeding, and pupation. Foliage was replaced every two days. Depending on availability, one to three single tree replicates, of each *Carpinus* and *Ostrya* taxon, were assayed with 10 individual larvae for a total of 10 to 30 larvae per taxon. The bioassay for a given larva was terminated at adult emergence. Larval longevity was determined as the difference in the days from the date the larva was first introduced to the foliage until pupation or death. Larval development time was the difference in days from initial introduction to the foliage until pre-pupation. Within 12 hours of pupation, each individual pupa was weighed (nearest 0.001 g) to obtain the pupal fresh weight. Gypsy moth pupae were held at room temperature until adult emergence or until the pupa was determined to be dead due to lack of movement following repeated prodding with a probe. The proportion of larvae reaching pupation was calculated by recording the total number of larvae that pupated in each of the containers for all single tree replicates (1 to 3) for a given taxon. At the termination of the no-choice larval feeding bioassay trial,

the fecal pellets from each of the containers for each taxon were dried in an oven at 50 °C and then weighed (nearest 0.001 g).

Multiple-choice (MC) laboratory larval feeding bioassay. The larval multiple-choice laboratory feeding bioassays were conducted as previously described by Miller et al. (2001a). For each of the three multiple-choice bioassays, ten first instar gypsy moth larvae were placed into each of 10 plastic petri dishes (0.6 × 15.0 cm). Each petri dish served as a replicate. Three leaf discs, 2.54 cm in diameter, representing one of each of the different *Carpinus* taxon choices, were placed into each dish and randomly arranged around the perimeter. The larvae had access to all foliage discs. The petri dishes were placed in clear plastic bags to prevent drying of the leaf discs and were held in an incubator under a photoperiod of 16:8 (L:D) at ~25 °C. Condensation of water on the lid of the petri dish indicated a high relative humidity. The dishes were examined daily for three days. Each day, the foliage discs were removed from the dishes, replaced, and visually evaluated by two independent estimators, to the nearest 5%, and their estimates were averaged. The estimators used a defoliation template to determine the proportion of leaf tissue removed (Hall and Townsend 1987; Miller and Ware 1997, 1999a; Miller et al. 1999, 2001a, 2001b). New foliage discs were arranged randomly around the perimeter of each dish to eliminate possible bias.

Measuring *Carpinus* leaf toughness and thickness. Prior to using the leaves for the NC laboratory feeding trials, each leaf was measured for leaf thickness and inner and outer leaf toughness. Leaves were collected in the field as previously described. In the lab, leaf thickness was determined by measuring the leaf using a Vernier caliper to the nearest micrometer. For outer leaf toughness, the pentrometer (Chatillon™ digital force meter, Greensboro, N.C.) was applied within 0.5 cm from the leaf edge; for inner toughness, the pentrometer was applied to the center of the leaf adjoining the mid-rib.

Measures of larval susceptibility, preference, and suitability. Measures of susceptibility for gypsy moth larvae was quantified by the percent of leaf tissue removed in the NC feeding bioassays and the amount of larval frass production (dry weight). Host preference was a function of the percent of leaf tissue removed in the MC feeding bioassays. Host suitability was determined by larval development time to pupation (days), percent of larvae pupating, pupal fresh weight (mg), and the percent of adult emergence. In order to further define host suitability for gypsy moth larvae, pupation, and adult emergence, we employed a suitability ranking system as described by Montgomery (1991), and summarized by Liebhold et al. (1995) by calculating the ratio of male and female pupal fresh weights for larvae feeding on *Carpinus* and *Ostrya* hosts evaluated in the NC feeding studies to the male and female pupal fresh weights (male = 0.339 g; female = 0.886 g) for larvae feeding on bur oak (*Q. macrocarpa*), a preferred standard. For example, in our study, the mean suitability ranking (SR) for *C. caroliniana* was calculated to be 0.65 (0.221 g/0.339 g) for male pupae and 0.68 (0.720 g/0.886 g) for female pupae (where the numerator is the male/female pupal fresh weight for larvae feeding on *C. caroliniana,* and the denominator is the male/female pupal fresh weight for larvae feeding on *Q. macrocarpa,* respectively).

Statistical Analysis. Measures of susceptibility, preference, and suitability for gypsy moth larvae were subjected to analysis of variance (ANOVA) using taxon as the main effect. Means of significant effect (5%) were compared with the Dunn's test. Coefficients of correlation were calculated by regression analysis for larval development time with percent pupation, percent adult emergence, pupal fresh weight, frass dry weight, leaf thickness, and leaf toughness; percent pupation was regressed with percent adult emergence; pupal fresh weight with frass dry weight, and leaf thickness and leaf toughness; and leaf thickness and toughness with mean frass dry weight. Host suitability rankings (SRs) were regressed with male and female pupal weights. The percent of leaf tissue removed, proportion of larvae pupating, and proportion of adults emerging for each taxon were arcsine transformed before analysis to correct for non-normality. All data are presented as original means ± SEM. Data were analyzed using the SigmaStat for Windows (Jandel Scientific 1992).

Results

Gypsy moth larval development time to pupation. A summary of mean larval development time to pre-pupation is presented in Table 1. Overall, there was no significant difference in mean larval development time to pre-pupation with mean male and female larvae taking 46 and 47 days, respectively. Larvae failed to pupate when feeding on *C. coreana, C. fargesii, C. orientalis*, and the hybrid *C. caroliniana × C. orientalis*, living a mean of 11 days (range = 5 to 23 days). Male larvae feeding on *C. betula, C. turczanianari, O. japonica, Q. macrocarpa* (reference species), and the hybrids of *C. caroliniana × C. cordata*, and *C. caroliniana × C.*

Table 1. Gypsy moth mean larval development, percent pupation, percent adult emergence, mean male and female pupal weights, and frass dry weights when feeding on selected *Carpinus* and *Ostrya* taxa.

Taxa	N[1]	Mean Larval Development[2,3] (days)	Mean % Pupation Male	Mean % Pupation Female	Mean % Adult Emergence Male	Mean % Adult Emergence Female	Mean Pupal Fresh Weights Male (g)	Mean Pupal Fresh Weights Female (g)	Mean Dry Frass Wt. (g)
Carpinus betulus	30	43±2.9b	27±0.08ab	43±0.09b	20±0.07a	37±0.09ab	0.319±0.028ab	0.720±0.078ab	11.391±1.1b
C. caroliniana	10	45±1.3b	10±0.06a	10±0.06a	3±0.03a	13±0.06a	0.221±0.054a	0.606±0.060ab	17.350±1.6b
C. causcisa	30	46±2.4b	40±0.07b	50±0.09ab	27±0.08a	37±0.09ab	0.342±0.013b	0.776±0.047ab	13.669±1.2b
C. cordata	20	34±6.3ab	17±0.07a	10±0.06a	10±0.07a	10±0.06ab	0.267±0.047ab	0.609±0.172ab	6.260±6.3a
C. coreana	10	5±0.0a	0±0.00a	0±0.00a	0±0.00a	0±0.00a	0.000±0.0a	----	----
C. fargesii	10	8±0.0a	0±0.00a	0±0.00a	0±0.00a	0±0.13a	0.000±0.0a	----	----
C. japonica	20	41±0.4b	20±0.13a	20±0.13a	20±0.13a	20±0.13ab	0.260±0.0a	----	7.284±6.9a
C. laxiflora	10	18±0.0ab	25±0.11a	25±0.13a	20±0.13ab	20±0.10ab	0.286±0.062ab	0.693±0.241ab	13.698±1.2b
C. orientalis	10	23±0.0a	0±0.00a	0±0.00a	0±0.00a	0±0.00a	0.000±0.0a	----	----
C. tschonoskii	20	44±4.2b	45±0.11b	40±0.11b	35±0.11a	30±0.10ab	0.355±0.075b	0.555±0.079a	19.826±2.0b
C. turczaninari	20	42±3.1b	35±0.11ab	10±0.07a	30±0.11a	5±0.05a	0.322±0.015b		13.668±2.1b
Ostrya carpinifolia	20	54±3.1b	40±0.11b	25±0.10ab	35±0.11a	25±0.10ab	0.258±0.020a	0.785±0.052ab	14.516±1.6b
O. japonica	20	38±3.2b	50±0.12b	25±0.10ab	30±0.11a	20±0.09ab	0.332±0.031b	0.907±0.061b	9.162±1.0ab
O. virginiana	10	34±0.6ab	10±0.10a	20±0.13ab	10±0.10a	20±0.13ab	-----	-----	7.566±8.1a
C. betulus x tschonoskii	30	45±2.6b	43±0.09b	47±0.09b	30±0.09a	37±0.09ab	0.290±0.020ab	0.700±0.058ab	3.852±0.3a
C. caroliniana x betulus	30	51±3.6b	30±0.09ab	37±0.09ab	23±0.08a	30±0.09ab	0.304±0.024ab	0.572±0.049a	10.780±1.0ab
C. carol. x (betulus x tschonoskii)	30	45±4.1b	43±0.09b	30±0.09ab	17±0.07a	30±0.09ab	0.227±0.016a	0.523±0.086a	13.334±1.2b
C. caroliniana x cordata	30	48±3.9b	40±0.09b	27±0.08ab	30±0.09b	27±0.08ab	0.299±0.020ab	0.673±0.107ab	18.110±2.0b
C. caroliniana x coreana	30	44±0.6b	20±0.07a	27±0.08ab	20±0.07a	27±0.08ab	0.257±0.017a	0.718±0.044ab	11.924±1.5b
C. caroliniana x laxiflora	20	40±4.6b	17±0.08a	7±0.07a	13±0.08a	7±0.07a	0.235±0.028a	-----	6.589±1.0a
C. caroliniana x orientalis	30	6±0.0a	0±0.00a	0±0.00a	0±0.00a	0±0.00a	-----		----
C. caroliniana x tschonoskii	30	38±0.6b	46±0.09b	7±0.05a	46±0.09a	7±0.05a	0.236±0.027a	0.457±0.001a	15.047±1.4b
'C. cordata x japonica	20	34±0.7ab	13±0.08a	7±0.07a	13±0.08ab	7±0.07a	0.262±0.031ab	0.509±0.079a	10.417±1.3ab
Quercus macrocarpa	30	57±2.7b	30±0.09ab	70±0.09b	30±0.09ab	70±0.09b	0.339±0.040b	0.886±0.051b	9.213±ab
Significance:		**F=109.5** P<0.001	**F=34.6** P=0.016	**F=67.7** P<0.001	**F=111.8** P<0.001	**F=60.7** P<0.001	**F=34** P<0.001	**F=2.7** P=0.002	**F=3.9** P<0.001

[1]N = Number of single petri dish replicates per *Carpinus* taxon
[2]Means followed by the same letter are not significantly different (Dunns' multiple comparison test; P<0.05)
[3]Larval development (days) is the time from when the larvae were introduced to the *Carpinus* foliage until pre-pupated
[4]Mean percent pupation is the percent of original gypsy larvae pupating
[5]Mean percent adult emergence is the percent of original larvae emerging as adults

laxiflora completed pupation in significantly fewer days (mean = 56 days; range = 51 to 59 days) compared to larvae feeding on *C. caroliniana, C. tschonoskii*, and the hybrid *C. caroliniana × C. tschonoskii* (mean = 66 days; range = 65 to 67 days) (*F* = 31.1; *P* = 0.04). Larvae feeding on the remaining *Carpinus* and *Ostrya* taxa were intermediate in completing pupation (mean = 60 days; range = 54 to 66 days). Female larvae, feeding on *O. japonica, O. virginiana*, and the reference species, *Q. macrocarpa* completed pupation in a significantly shorter time (mean = 54 days; range = 51 to 56 days) compared to larvae feeding on *C. japonica, C. turczamoamari.*, and the hybrid *C. caroliniana × C. laxiflora* taking a mean of 72 days (range = 71 to 73 days) (*F* = 2.8; *P* < 0.001). Female larvae feeding on the remaining taxa were intermediate in completing pupation (mean = 62 days; range = 57 to 67 days).

Frass production. Larval frass dry weight production is summarized in Table 1. Larvae feeding on *C. betulus, C. caroliniana, C. causcica, C. laxiflora, C. tschonoskii,, C. turczaninari, and O. carpinifolia,* and the hybrids of *C. caroliniana × C. cordata, C. caroliniana × C. coreana,* and *C. caroliniana × C. tschonoskii* produced significantly more frass (mean = 14.5 g; range = 11 to 18 g) compared to larvae feeding on *C. cordata, C. japonica, O. virginiana*, and the hybrids of *C. betula × C. tschonoskii, C. caroliniana × C. laxiflora* with less than 7.4 g of frass produced (*F* = 3.9; *P* < 0.001). Larvae feeding on the other *Carpinus* and *Ostrya* taxa and the reference species, *Q. macrocarpa* produced intermediate amounts of frass (mean = 10.1 g; range 9.162 to 10.417 g). Larvae feeding on *C. coreana, C. fargesii, C. orientalis*, and the hybrid *C. caroliniana × C. orientalis* did not produce any frass (Table 1). The time for larval development to pupation was not reflective of frass production, (r^2 = 0.09; *P* = 0.15) and frass production was not a good predictor of pupal fresh weight (r^2 = 0.10; *P* = 0.19).

Mean pupal fresh weight. Male and female gypsy moth pupal fresh weights are summarized in Table 1. For all *Caprinus* and *Osytra* taxa, male pupae had significantly lower mean pupal fresh weights (0.293 g) compared to female pupae (0.727 g) (*t* = 22287; *P* < 0.001). Male pupal weight was significantly higher for larvae feeding on *C. causisca, C. tschonoskii, C. turczamamari, O. japonica,* and the reference species, *Q. macrocarpa* compared to larvae feeding on *C. caroliniana, C. japonica, O. carpinifolia,* and the hybrids *C. caroliniana × (C. betula × C. tschonoskii), C. caroliniana × C. coreana, C. caroliniana × C. laxiflora*, and *C. caroliniana × C. tschonoskii* (*F* = 35.4; *P* = 0.01). The remaining *Carpinus* taxa had intermediate

pupal fresh weights. Female pupal weights were significantly higher for larvae feeding on the preferred host of *Q. macrocarpa* (0.886 g) and the highly susceptible *O. japonica* (0.907 g) compared to larvae feeding on *C. tschonoskii* (0.555 g) and the hybrids *C. caroliniana × C. betula* (0.572 g) *C. caroliniana × (C. betula × C. tschonoskii)* (0.227 g), *C. caroliniana × C. tschonoskii* (0.457 g) and *C. cordata × C. japonica* (0.509 g) (*F* = 2.7; *P* = 0.002). Female pupal weights for larvae feeding on the remaining *Carpinus* and *Ostrya* taxa had intermediate pupal weights. There was no relationship (r^2 = 0.0; *P* = 0.94) between larval development time to pupation and pupal fresh weight.

Pupation and adult emergence. Mean percent gypsy moth pupation and adult emergence is summarized in Table 1. For all *Carpinus* and *Ostyra* taxa, there was no significant difference in mean male percent pupation (28%) versus female pupation (27%). Male larvae feeding on a majority of the *Carpinus* and *Ostrya* taxa had significantly higher percent pupation rates of (mean = 39%; range = 27% to 50%) compared to male larvae feeding on leaves of *C. coreana, C. fargesii, C. orientalis,* and the hybrid *C. caroliniana × C. orientalis* all of which failed to pupate (*F* = 6.1; *P* < 0.001). Male larvae feeding on *C. caroliniana, C. japonica, C. turczamamari* , and the hybrid *C. cordata × C. japonica* had intermediate percent pupation (mean = 11%; range 10 to 13%). Thirty percent of male larvae pupated when feeding on the reference host, *Q. macrocarpa*. Mean percent pupation for female larvae mirrored male pupation for larvae feeding on all three *Ostrya* taxa and eight *Carpinus* taxa with significantly higher mean percent pupation (mean = 33%; range = 20% to 47%) compared to remaining taxa (Table 1). Larval development time to pupation was a strong predictor of percent pupation (r^2 = 0.83; *P* < 0.001).

There was no significant difference in percent male versus percent female adult emergence (23% versus 24%) with a male to female ratio of 1.2. Adult emergence was strongly correlated with both larval development time to pupation (r^2 = 0.90; *P* < 0.001) and percent pupation (r^2 = 0.83; *P* < 0.001) with adults emerging on all of the *Carpinus* and *Ostrya* taxa tested (Table 1).

Multiple-choice larval feeding preference bioassay. A summary of the three gypsy moth larval multiple-choice (MC) studies is presented in Table 2. In MC #1, gypsy moth larvae preferred *C. betulus* over *C. coreana* and *C. fargesii* (24%, 20% and 6% leaf tissue removed, respectively) (*F* = 19.6; *P* < 0.001). *Carpinus caroliniana* was highly preferred over *C. coreana* and

Table 2. Multiple-choice studies for gypsy moth larvae feeding on *Carpinus* taxa (2006).

Taxa	Mean % Leaf Tissue Removed[1]
MC-1	
C. betulus	24±5.3b
C. coreana	20±0.8a
C. fargesii	6±2.5a
Significance:	**F=19.6 P<0.001**
MC-2	
C. caroliniana	26±3.8c
C. coreana	8±1.6b
C. fargesii	3±1.0a
Significance:	**F=28.7 P<0.001**
MC-3	
C. caroliniana	15±2.7b
C. orientalis	15±3.7b
C. caroliniana x *C. orientalis*	2±0.8a
Significance:	**F=20.3 P<0.001**

[1]Values within columns followed by the same letter are not significantly different (P<0.05; Dunn's test)

C. fargesii in MC #2 (26%, 8%, and 3%, respectively) ($F = 28.7$; $P < 0.001$). In MC #3, the hybrid *C. caroliniana × C. orientalis* was the least preferred (2% leaf tissue removed) compared to *C. caroliniana* and *C. orientalis* alone, both with 15% leaf tissue removed ($F = 20.3$; $P < 0.001$) (Table 2). In MC studies #1 and #2, feeding preference and susceptibility (NC studies) were highly correlated. In MC study #1, gypsy moth larvae preferred the susceptible *C. betula* over the less susceptible *C. coreana* and *C. fargessi* ($r^2 = 0.99$; $P = 0.04$), and in MC study #2, the more susceptible *C. caroliniana* was much more preferred over the less susceptible *C. cordata* and *C. fargesii* ($r^2 = 0.99$; $P = 0.04$). No such relationship was found in MC study #3, for gypsy moth larvae, when given a choice, between *C. caroliniana*, *C. orientalis*, and the hybrid *C. caroliniana × C. orientalis*.

***Carpinus* leaf thickness and toughness.** A summary of leaf thickness and inner and outer leaf toughness of *Carpinus* taxa is presented in Table 4. *Carpinus betulus, C. caroliniana, C. orientalis, C. turczaninovii*, and the hybrids of *C. caroliniana × C. cordata, C. caroliniana × C. coreana, C. caroliniana × C. orientalis, C. cordata × C. japonica* had significantly thicker leaves while *C. japonica* and *C. tschonoskii* had the thinnest leaves ($F = 116.6$; $P < 0.001$). Leaves of the hybrids of *C. betulus × (C. betulus × C. tschonoskii)*, and *C. caroliniana ×*

Table 3. Suitability rankings (SRs) for selected *Carpinus* and *Ostrya* taxa for male and female gypsy moth pupae compared to the reference species, *Quercus macrocarpa*.

Taxa	Male Pupal Suitability Ranking[1]	Female Pupal Suitability Ranking[1]
Carpinus betulus	0.941±0.0829ab	0.813±0.0888ab
C. caroliniana	0.651±0.158a	0.684±0.0680ab
C. causcisa	1.009±0.0376b	0.876±0.0532ab
C. cordata	0.789±0.138ab	0.688±0.194ab
C. japonica	0.767±0.0257ab	----[2]
C. laxiflora	0.844±0183ab	0.782±0.272ab
C. tschonoskii	1.047±0.221b	0.626±0.0892a
C. turczaninari	0.951±0.0458ab	----[2]
Ostrya carpinifolia	0.760±0.0584ab	0.709±0.183ab
O. japonica	0.978±0.0912ab	1.023±0.0693b
C. betulus x *tschonoskii*	0.641±0.120a	0.790±0.0660ab
C. caroliniana x *betulus*	0.896±0.0726ab	0.646±0.0558a
C. carolinian x *(betulus* x *tschonoskii)*	0.670±0.0489ab	0.590±0.0971a
C. caroliniana x *cordata*	0.882±0.0594ab	0.633±0.160a
C. caroliniana x *coreana*	0.758±0.0514ab	0.810±0.0512ab
C. caroliniana x *laxiflora*	0.693±0.0828ab	----[2]
C. caroliniana x *tschonoskii*	0.697±0.0788ab	0.516±0.00113a
C. cordata x *japonica*	0.773±0.0919ab	0.574±0.0892a
Quercus macrocarpa	0.999±0.119b	1.000±0.0572b
Significance:	**F=1.7, P=0.049**	**F=2.5, P=0.004**

[1]Values within columns followed by the same letter are not significantly different (P<0.05; Dunn's test)

[2]Larvae failed to reach pupation

Table 4. Summary of leaf thickness (microns), and inner and outer leaf toughness (grams) of selected *Carpinus* taxa.

Taxa[1]	Thickness (microns)	Toughness (grams) Inner	Outer
Carpinus betulus	250.00±25.0d	17.8286±5.9b	19.5657±1.0b
Carpinus betulus × (*C. betulus* × *C. tschonoskii*)	145.00±13.0ab	16.3657±4.8ab	14.4457±2.1ab
Carpinus betulus × *C. tschonoskii*	180.00±18.0b	19.5657±5.7bc	17.0057±2.5ab
Carpinus caroliniana	202.50±14.0c	17.3714±4.9b	15.0857±3.2ab
Carpinus caroliniana × (*C. betulus* × C. *tschonoskii*)	175.00±17.5b	15.2686±6.1ab	13.8057±1.4ab
Carpinus caroliniana × *C. cordata*	202.50±40.2c	16.2743±5.2ab	16.9143±1.5ab
Carpinus caroliniana × *C. coreana*	222.50±44.1c	21.5771±5.6bc	23.3143±2.1c
Carpinus caroliniana × *C. laxiflora*	162.50±10.7b	16.6857±1.2ab	15.0400±1.2ab
Carpinus caroliniana × *C. orientalis*	212.50±11.0c	48.8343±14.6c	45.0743±5.4d
Carpinus carolinana × *C. tschonoskii*	190.00±9.5b	16.4571±4.2ab	14.7200±1.6ab
Carpinus cordata	145.00±11.6ab	21.0286±8.6bc	15.8171±2.1ab
Carpinus cordata × *C. japonica*	170.00±13.6b	16.0914±4.1ab	14.7200±2.0ab
Carpinus coreana	207.50±22.1c	19.4743±5.2bc	19.2000±1.8b
Carpinus fargesii	180.00±10.8b	19.8400±5.3ab	18.5600±1.6ab
Carpinus japonica	117.50±8.9a	9.9657±2.1a	8.96000±1.0a
Carpinus laxiflora	125.00±6.2ab	16.0000±4.0ab	14.9943±1.3ab
Carpinus orientalis	207.50±21.6c	27.8857±8.7bc	29.9886±3.2bc
Carpinus tschonoskii	157.50±10.9ab	16.9143±4.8ab	17.0057±1.6ab
Carpinus turczaninovii	207.50±21.1c	17.0057±4.2ab	15.3600±1.6ab
Significance:	**F=116.6 P<0.001**	**F=62.2 P<0.001**	**F=68.1 P<0.001**

[1]Values within columns followed by the same letter are not significantly different (P<0.05; Dunn's test)

C. laxiflora, and the species, *C. cordata*, and *C. laxiflora* were intermediate in thickness. Inner leaf portions of the hybrids *C. betulus* × *tschonoskii*, *C. caroliniana* × *coreana*, *C. caroliniana* × *orientalis,* and species, *C. betulus* and *C. cordata*, were significantly tougher than the inner leaf portions of *C. japonica* leaves. Inner leaf toughness for the other taxa was intermediate (F = 62.2; $P < 0.001$). Outer leaf toughness was less variable with only the leaves of the hybrids *C. caroliniana* × *cordata* and *C. caroliniana* × *orientalis* being significantly tougher than *C. japonica* (F = 68.1; $P < 0.001$). Outer leaf toughness for the remaining *Carpinus* taxa was intermediate (Table 4). Leaf thickness was correlated with outer leaf toughness (r^2 = 0.25; P = 0.03), but was not related to inner leaf toughness.

Effect of leaf thickness and toughness on the susceptibility and suitability of *Carpinus* taxa to larval feeding. Larval development time to pre-pupation was not correlated with *Carpinus* leaf thickness (r^2 = 0.03; P=0.67) but was related to inner (r^2 = 0.30; P = 0.03) and outer leaf thickness (r^2 = 0.30; P = 0.03). Further, mean pupal fresh weight and leaf thickness (r^2 = 0.12, P=0.18) were not related, but pupal fresh weight was related to inner (r^2 = 0.31, 0.56; P = 0.04) and outer leaf toughness (r^2 = 0.31, 0.56; P = 0.04). Leaf thickness and outer leaf toughness do not appear to be good predictors of frass production (thickness = r^2 = 0.20; P = 0.08; outer toughness = r^2 = 0.18; P = 0.10), but frass production was related to inner leaf toughness (r^2 = 0.24; P = 0.05).

Suitability Rankings (SRs). Suitable rankings (SRs), for male and female gypsy moth larvae feeding on *Carpinus* and *Ostyra* taxa, are summarized in Table 3. For all taxa tested, the overall mean male SR was 0.85 (range = 0.64 to 1.05) and 0.77 (range = 0.52 to 1.02) for female pupae, indicating that the majority of the *Carpinus* taxa tested in this study have a moderate to high host suitability for gypsy moth larvae. The SRs for *C. caroliniana* (male = 0.66 and female = 0.68), and for *O. virginiana* (male = 0.76 and female = 0.95) were within Liebhold's et al. (1995) rating of 2 (SR range = 0.5 to 0.9) for a marginal to intermediate host (i.e. *C. caroliniana),* and a rating of 1.0 (SR greater than 0.9) for an intermediate to highly preferred host (i.e. *O. virginiana*). Host suitability significantly affected male and female pupal weights differently (t = 22287.000; P = <0.001). Overall, female pupae were approximately 2.5 times heavier than male pupae (male = 0.293 g versus female = 0.721 g**).** Our findings, for these two taxa, are consistent with results reported by Mosher

(1915), Mauffette et al. (1983), Montgomery (1991), Twery (1991)**,** Liebhold et al. (1995), and Fite (2019). *Carpinus causcica* (SR = 1.00) and *C. tschonoskii* (SR = 1.05), and the reference species, *Q. macrocarpa* (SR = 1.00) were significantly more suitable hosts for male larval development compared to male larvae feeding on *C. caroliniana* (SR = 0.65), and the hybrid *C. betulus × C. tschonoskii* (SR = 0.64). All of the additional *Carpinus* and *Osytra* taxa (SR range = 0.67 to 0.98) were intermediately suitable for male gypsy moth larvae. *Ostrya japonica* (SR = 1.02) and the reference species, *Q. macrocarpa* (SR = 1.00) were significantly more suitable for female larval development compared to *C. tschonoskii* (SR = 0.63), and the hybrids, *C. caroliniana × C. betula* (SR = 0.65), *C. caroliniana × (C. betula × C. tschonoskii)* (SR = 0.59), *C. caroliniana × C. cordata* (SR = 0.63), *C. caroliniana × C. tschonoskii* (SR = 0.52), and *C. cordata × C. japonica* (SR = 0.57). All of the additional *Carpinus* and *Osytra* taxa (SR range = 0.68 to 0.81) were intermediately suitable for female gypsy moth larval development (F = 2.5; P = 0.004) (Table 3). Both male and female pupal weights were strong predictors of suitability (male: r^2 = 0.88; P < 0.001; female: r^2 = 0.87; P < 0.001).

Discussion

In this study, we examined the relative susceptibility, preference, and suitability of *Carpinus* and *Ostrya* taxa for feeding and development by gypsy moth larvae, which will hopefully provide new insight into the potential use of these taxa in an overall tree breeding program.

Gypsy moth larvae feed on a wide variety of woody plant hosts. Variation in host susceptibility, preference, and suitability can be quite variable across and within genera (Mosher 1915, Barbosa and Capinera 1977, Barbosa and Greenblatt 1979, Mauffette et al. 1983, Lechowicz and Mauffette 1986, Berisford et al. 1990, Montgomery 1991, Liebhold et al. 1995, Foss and Rieske 2003, Miller 2008, Pearse 2011, Fite 2019).

Plants protect themselves from herbivores in one of two ways; by using either physical plant traits, chemical defenses, and/or a combination of both. In all likelihood, that is the case here with *Carpinus* and *Ostrya* taxa (Hoxie et al. 1975; Meredith and Schuster 1979; Johnson et al. 1980a,b; Ryan et al. 1982; Matsuda and Senbo 1986; Tingey and Laubengayer 1986; Doss et al. 1987; Potter and Kimmerer 1989; Ranney and Walgenbach 1992; Spicer et al. 1995; Patton et al. 1997; Fulcher et al. 1998; Rowe and Potter 2000; Dalin and Bjorkman 2003). Gypsy moth host plant leaf chemistry has been widely studied on a variety of plant

hosts, and undoubtedly plays a major role in host plant defense against the gypsy moth (Thorsteinson 1960; Barbosa and Greenblatt 1979; Mattson 1980; Scriber and Slansky 1981; Schultz et al. 1982; Martinat and Barbosa 1987; Mattson and Scriber 1987; Schultz 1988; Foss and Rieske 2003; Rieske et al. 2003; Paluch et al. 2008, 2009), but was beyond the scope of this study. However, here we will discuss physical plant characteristics (i.e. leaf thickness, toughness, and pubescence) of *Carpinus* leaves, and their possible role in relative host susceptibility, preference, and suitability for gypsy moth larvae.

Relative susceptibility, preference and suitability of *Carpinus* taxa for gypsy moth larval development. The acceptability of a host plant by a herbivore is considered an indicator of host susceptibility and suitability (Martinat and Barbosa 1987, Montgomery 1994). In our study, host susceptibility was reflective of host suitability (r^2 = 0.50; P < 0.001). Using larval frass production as a measure of feeding susceptibility, the vast majority of the taxa tested here appear to be moderately to highly susceptible to feeding by gypsy moth larvae; exceptions being *C. coreana*, *C. fargesii*, and *C. orientalis* and the hybrid *C. caroliniana × C.orientalis* where larvae failed to produce any measurable frass.

Physical plant traits, such as leaf toughness and thickness, can be main factors affecting invertebrate feeding, and usually correlate with leaf fiber and lignin content (Tanton 1962, Graca and Zimmer 2005). Agrawal and Fishbein (2006) found leaf toughness could be used to predict herbivory of many plants including milkweeds (*Asclepias* spp.). Leaf thickness and toughness may also play a role in the relative susceptibility, preference, and suitability of *Carpinus* and *Osytra* taxa for feeding by and development of gypsy moth larvae. Pearse (2011) found that for leaf-feeding caterpillars, oak leaf toughness was one of the best predictors of tussock moth survival along with water content, and condensed and total tannin content. He also found a negative correlation between caterpillar survival and leaf toughness. Conversely, Keathley and Potter (2008) found that fall webworm larvae preferred the thicker and tougher leaves of lilac (*Syringa vulgaris*), Bradford pear (*Pyrus calleryana* 'Decaisne'), dogwood (*Cornus florida*), and tuliptree (*Lirodendron tulipfera*) over the thinner, and more tender leaves of sassafras (*Sassafras albidum*) and little leaf linden (*Tilia cordata*). Our results are similar to Keathley and Potter (2008) in that inner and outer leaf toughness was positively correlated with gypsy moth larval longevity, but larval longevity was not cor-

related with leaf thickness. Additionally, in our multiple choice feeding bioassays, when given a choice, gypsy moth larvae tended to prefer the thicker and tougher leaves of *C. betulus*, *C. caroliniana*, *C. coreana*, and *C. orientalis* over the thinner and less tough *C. fargesii* leaves.

Larval survival has been a common criteria used to document host suitability, with high first instar survival indicating high suitability and low first instar larval survival suggesting poor host susceptibility (Barbosa and Krischik 1987; Martinat and Barbosa 1987; Miller and Hanson 1989 a,b). In our study, larval development time and survival were not correlated with leaf thickness, but were correlated with inner and outer leaf toughness. Larvae feeding on the tougher leaves of *C. coreana, C. orientalis*, and the hybrid *C. caroliniana × C. orientalis* only lived 7 to 22 days, failed to pupate, and produced little or no frass. Additionally, only 20% and 27% of larvae feeding on the tougher leaves of *C. caroliniana* and *C. cordata* pupated, respectively. *Carpinus orientalis* has thick and tough leaves, and is native to Eastern Europe and Asia Minor which most likely overlaps with the historical origins of the gypsy moth. Consequently, historical co-evolution may have contributed to the reduced suitability of *C. orientalis* as a host. Miller et al. (2001a) found that spring and fall cankerworm larvae feeding on the thicker and tougher Asian elm leaves lived a shorter period of time, had extended larval development time, failed to pupate or had low (less than 11% pupation) rates, and removed less than 15% of leaf tissue compared to larvae feeding on thinner and less tough North American elm taxa. However, co-evolution does not completely explain the lack of larval host suitability. For example, *C. caroliniana,* which is native to North America, has tough leaves and is considered to be an intermediate to moderate gypsy moth larval host (Mosher 1915; Montgomery 1991, 1994; Fite 2019). Conversely, *C. coreana*, which is native to Korea, also has tough leaves, but when given a choice was not preferred by gypsy moth larvae. In all likelihood, neither of these aforementioned plant species would have co-evolved with the gypsy moth, but would be expected to be somewhat suitable hosts.

Gypsy moth larvae generally complete development between 35–40 days on highly suitable hosts (Barbosa and Capinera 1977, Barbosa and Greenblatt 1979, Miller and Hanson 1989a). In our study, there was no significant difference in male and female larval development time to pupation (males = 46 days; females = 47 days) on suitable hosts; development time was well within the range for known moderate to highly suitable hosts (mean = 42 days; range = 34–51 days) (Barbosa and Capinera 1977, Barbosa and Greenblatt 1979, Miller and Hanson 1989a). In this study, larval development time to pupation was highly correlated with percent pupation and adult emergence (pupation: r^2 = 0.83; $P < 0.001$; adult emergence (r^2 = 0.81, $P < 0.001$), indicating that the longer the larvae fed the more likely they were to pupate and emerge as adults. Conversely, larvae feeding on unsuitable *Carpinus* hosts lived a mean of l3 days (range of 5-23 days) and none of the larvae completed larval development or pupated. However, longer larval development time to pupation did not necessarily reflect a higher pupal fresh weight or greater larval frass production, and frass production did not reflect pupal fresh weight. For example, larvae feeding on the highly preferred host, bur oak (*Q. macrocarpa*) took 43 days to reach the pupal stage, but produced significantly less frass (9.2 mg). Larvae feeding on the moderately suitable *C. carolinana* and *C. causcisa* hosts took a similar number of days (45 and 46 days, respectively) to reach the pupal stage, but produced significantly more frass. Consistent with our findings, Miller et al. (1991) found that larval development was not correlated with pupal fresh weight or frass production for gypsy moth larvae feeding on Garry oak (*Q. garryana*) and red alder (*Alnus rubra*). Additionally, Foss and Rieske (2003) found that bur oak (*Q. macrocarpa*) was the most preferred of five oak species tested, but gypsy moth larvae consumed relatively small amounts of foliage and developed rapidly. Bur oak leaves in their study were the toughest, contained the highest levels of carbohydrates and tannins, and only moderate levels of nitrogen. Similarly, Miller, F. (in prep) also found that gypsy moth larvae feeding on thicker and tougher bur oak leaves had a relatively shorter development time and lower frass production compared to six other common oak species tested. Results from this study and other gypsy moth suitability studies suggest that there is considerable variation either in the nutritional value and/or host plant chemistry of *Carpinus* and *Oystra* taxa which may significantly affect larval development, but not enough to prevent pupation.

It is well-known that leaf trichomes help protect plants from insect herbivores specifically as it relates to feeding, growth, survival, and oviposition (Pillemer and Tingey 1976, Ramalho et al. 1984, Fordyce and Agrawal 2001). Feeding may be negatively correlated with trichome density, which is generally considered a "soft weapon" in plant defense compared to other plant traits (Levin 1973; Danielson et al. 1987; Potter et al. 1998; Miller and Ware 1999a,b; Miller et al. 1999; Miller 2000; Miller et al. 2001a,b;

Dalin and Bjorkman 2003; Dalin et al. 2008). Plants have the ability to produce glandular (chemical-producing) and non-glandular trichomes. They may vary in morphology and genetics, and even within individual plant species (MacLean and Byers 1983; Southwood 1986; Agrawal 1999, 2000; Werker 2000; Dalin and Bjorkman 2003; Loe et al. 2007). Non-glandular trichomes function in structural defense and have low nutritional value while glandular trichomes provide both structural and chemical defense and may contain terpenes and alkaloids that act as feeding deterrents or toxins (Levin 1973, Rautio et al. 2002, War et al. 2012). Gypsy moth larvae feed by consuming the entire leaf, including leaf veins, in contrast with other insect herbivores, which skeletonize (i.e. Japanese beetle, *Popillia japonica* Newman), "windowpane" (i.e. leaf beetle larvae), or chew holes in the leaves (i.e. adult leaf beetles and flea weevils). In this study, pubescent and non-pubescent leaves were equally fed upon, indicating that leaf and veinal pubescence does not affect feeding susceptibility, preference, or suitability. For example, *C. japonica* and *C. tschonoskii* both have pubescent leaves, were moderately susceptible to larval feeding, and were highly suitable for larval development (Krussman 1976). Similarly, the leaves of *C. caroliniana, C. cordata, C. orientalis*, and *C. turczaniowii* all have thin pubescence on the veins and midrib on the leaf underside (Krussman 1976), and were also found to be moderately to highly susceptible and preferred, but not very suitable. Miller et al. (2001a) found, in multiple-choice bioassays feeding studies, that there was no consistent feeding preference pattern for leaf consuming spring and fall cankerworm larvae feeding on pubescent elm leaves.

Using the suitability rankings (SRs), as previously described by Montgomery (1991) and Liebhold et al. (1995), we found a strong correlation between both male and female pupal weights and host suitability, (male pupae: $r^2 = 0.87$; $P < 0.001$; female pupae: $r^2 = 0.88$; $P < 0.001$). Suitability appears to have a significant effect on both male and female pupal weights with females weighing nearly 2.5 times more than males. A mean suitability ranking (SR) of 0.85 for male pupae (range = 0.64 to 1.05) and female pupae 0.77 (range = 0.52 to 1.02), for all taxa, indicate that the vast majority of *Carpinus* and *Ostrya* taxa have moderate to high host suitability for gypsy moth larvae. More specifically, our SRs for male and female gypsy moth pupae for *C. caroliniana* (male = 0.66; female = 0.68) and SRs of 0.76 (male) and 0.95 (female) for *O. virginiana,* are comparable to suitability rankings identified by Liebhold et al. (1995) suggesting *C.*

caroliniana represents an intermediate host and *O. virginiana* an intermediate to highly preferred host (Mosher 1915, Montgomery 1991, Twery 1991, Liebhold et al. 1995, Fite 2019).

Hybridization, feeding susceptibility and preference, and suitability of *Carpinus* taxa for gypsy moth larvae. In this study, it appears that hybridization may have a either a stabilizing, positive, or negative influence on host susceptibility, preference, and suitability for gypsy moth larvae, may be polygenic, and may not be the result of single gene expression (Paige and Capman 1993, Orions 2000, Cheng et al. 2011). It is well known that secondary metabolites are a common chemical defense employed by plants against herbivorous insects and hybridization may increase the variation of secondary metabolites affecting herbivore resistance (Rieseberg and Ellstrand 1993, Patton et al. 1997, Fulcher et al. 1998, Orians 2000, Cheng et al. 2011). Further, most secondary metabolites (SMs) in hybrids may also be present in the parents, but hybrids may also miss some parental secondary metabolites or have novel ones (Paige and Capman 1993, Cheng et al. 2011, Lopez-Caamal and Tovar-Sanchez 2014). Hybridization appeared to have a stabilizing effect on the moderate suitability of the individual species of *C. betulus*, *C. caroliniana*, *C. cordata*, and *C. tschonoskii* and their associated hybrids of *C. betulus × tschonoskii*, *C. carolinana × betulus*, *C. caroliniana × (betulus × tschonoskii*, and *C. caroliniana × cordata)* which were also moderately suitable for gypsy moth larval development. Further, when the moderately suitable *C. caroliniana* (SR = 0.65) was hybridized with the unsuitable (SR = 0, no larvae reached pupation) species of *C. coreana,* the SR for the resulting hybrid *(C. caroliniana × C. cordata)* increased (SR = 0.81). In contrast, when the highly preferred (SR = 0.80) *C. cordata* was hybridized with the unsuitable *C. japonica* (SR = 0), the SR value for the hybrid (*C. cordata × C. japonica*) decreased (SR = 0.57). Apparently, the unsuitable traits associated with *C. coreana* were not preserved in the hybrid leading to higher suitability, but were preserved with *C. japonica* resulting in lower suitability. More dramatically, when *C. caroliniana* was crossed with the unsuitable species of *C. orientalis* (SR = 0), the resulting *C. caroliniana × C. orientalis* hybrid was also unsuitable (SR = 0) for gypsy moth larval development. These findings are further supported in multiple-choice study #3 (MC-3), where, when given a choice, gypsy moth larvae removed significantly more leaf tissue from *C. caroliniana* and *C. orientalis* leaves compared to the *C. caroliniana × C. orientalis* hybrid. In contrast, hybridization

rendered the *C. caroliniana* × *C. laxiflora* hybrid unsuitable compared to the moderately suitable parents of *C. carolinana* (SR = 0.65) and *C. laxiflora* (SR = 0.79).

In conclusion, based on the finding in this study, there does not appear to be a large pool of *Carpinus* or *Ostrya* taxa suitable for future tree breeding programs and for use in areas where gypsy moth outbreaks are frequent. However, there are a few *Carpinus* taxa (i.e. *C. coreana, C. fargesii, C. orientalis*, and the hybrid *C. carolinana* × *C. orientalis*) on which gypsy moth larvae were not able to complete development, pupate, or reach the adult stage. The genera of *Carpinus* and *Ostrya* consist of a number of small to medium-sized deciduous trees which are distributed across the temperate regions of the northern hemisphere. Most are slow growing and can tolerate a variety of soil conditions (i.e. moisture, pH, and texture), and require minimal maintenance (i.e. pruning) making them good candidates for use in urban settings as hedges, screening and group plantings, and parkway and landscape trees particularly in areas where chronic gypsy moth feeding pressure is absent or rare, reducing the need for intensive chemical protection (Krussman 1976, Raupp et al. 1992, Dirr 2009, Sjoman et al. 2019). In order to make better use of the genotypic and phenotypic attributes of *Carpinus* and *Osytra* taxa in urban landscapes, where chronic insect defoliation is common, further studies are needed to determine the factor(s) responsible for feeding susceptibility and preference, and host suitability of *Carpinus* and *Ostrya* taxa for gypsy moth larvae.

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